Understanding Trabecular Meshwork Physiology: A Key to the Control of Intraocular Pressure?

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The trabecular meshwork is a tissue located in the anterior chamber angle of the eye, and it is a crucial determinant of intraocular pressure values because of its resistance to the evacuation of aqueous humor from the eye. Here we bring together classical and recent discoveries on the function of the trabecular meshwork, keys to understanding eye pathophysiology.

The anterior and posterior chambers of the eye are filled with aqueous humor, a fluid with an ionic composition very similar to the blood plasma and with two main functions: to supply nutrients to the avascular structures of the eye, cornea, and lens and to maintain intraocular pressure (IOP) within its physiological range, because it is the volume of aqueous humor in the eye chambers that determines the IOP value. Both nutrition of lenses and maintenance of IOP are critical for a normal visual process.

Aqueous humor undergoes a constant turnover. Initially, aqueous humor is secreted to the posterior chamber of the eye by the ciliary processes of the ciliary body in a process known as aqueous humor inflow. The mechanisms of aqueous humor inflow have been well documented and combine ultrafiltration from arterial blood and active secretion. Aqueous humor then reaches the anterior chamber by crossing the pupil and exchanges its contents with cornea and lens. Finally, aqueous humor is recycled by exiting the eye and returning to systemic circulation in a process defined as aqueous humor outflow (Fig. 1). Outflow rate is then one of the two elements of the equation defining aqueous humor volume and, thereby, IOP value.

There are two different pathways of aqueous humor outflow, both located in the iridocorneal angle of the eye (Fig. 1). The uveoscleral or nonconventional pathway refers to the aqueous humor leaving the anterior chamber by diffusion through intercellular spaces among ciliary muscle fibers [reviewed by Bill (2)]. Although this seems to be a minority outflow pathway in humans, the uveoscleral or nonconventional pathway is the target of specific antiglaucoma drugs (latanoprost, a prostaglandin $F_{2\alpha}$ analog) that increase the functionality of this route. In the human eye, the main outflow route is the trabecular or conventional outflow pathway. On this route, aqueous humor exits the eye through a well-structured tissue called the trabecular meshwork (TM). After crossing the TM, aqueous humor reaches Schlemm's canal (Fig. 1), which drains directly to the aqueous veins. Aqueous humor outflow via the trabecular pathway is IOP dependent, usually measured as outflow facility, and expressed in microliters per minute per millimeter of mercury.

Among glaucomas, most of those known as open-angle glaucomas (≈85% of total glaucomas) are caused by an increase in the resistance to aqueous humor drainage through the TM and/or Schlemm's canal. Sequentially, first the outflow facility decreases and then, as a consequence, aqueous humor volume and IOP increase.

However, many aspects of the regulation of aqueous humor outflow remain unclear. This fact is evidenced by the great number of drugs used for the treatment of open-angle glaucoma. The drugs most commonly used to treat open-angle glaucoma either decrease the production of aqueous humor in the ciliary body or increase the uveoscleral outflow. Drugs acting directly on the TM have not yet been developed, probably because the mechanisms governing the function of this tissue are just beginning to be elucidated. However, due to the quantitative significance of this structure in the drainage of aqueous humor, it can be anticipated that future antiglaucoma therapy will be directed toward the increase of trabecular outflow.

Anatomy of the TM: different regions with different functions

In humans, the majority of aqueous humor exits the eye via the TM (conventional or trabecular pathway). This tissue contains three differentiated layers (Fig. 2). From the inner to the outermost part, the layer of tissue closest to the anterior chamber is the uveal meshwork, formed by prolongations of connective tissue arising from the iris and ciliary body stromas totally covered by endothelial cells. This layer does not offer much resistance to aqueous humor outflow because intercellular spaces are large. The next layer, known as the corneoscleral meshwork, is characterized by the presence of lamellae covered by endothelium-like cells standing on a basal membrane. The lamellae are formed by glycoproteins, collagen, hyaluronic acid, and elastic fibers. The higher organization of the corneoscleral meshwork in relation to the uveal meshwork as well as their narrower intercellular spaces are responsible for the increase in flow resistance. The third layer, which is in direct contact with the inner wall of endothelial cells from Schlemm's canal, is the juxtacanalicular or cribriform meshwork (Fig. 2). It is formed by cells embedded in a dense extracellular matrix, and the majority of the tissue resistance to flow supposedly lies in this layer, due to its narrow intercellular spaces. The layer of endothelial cells from

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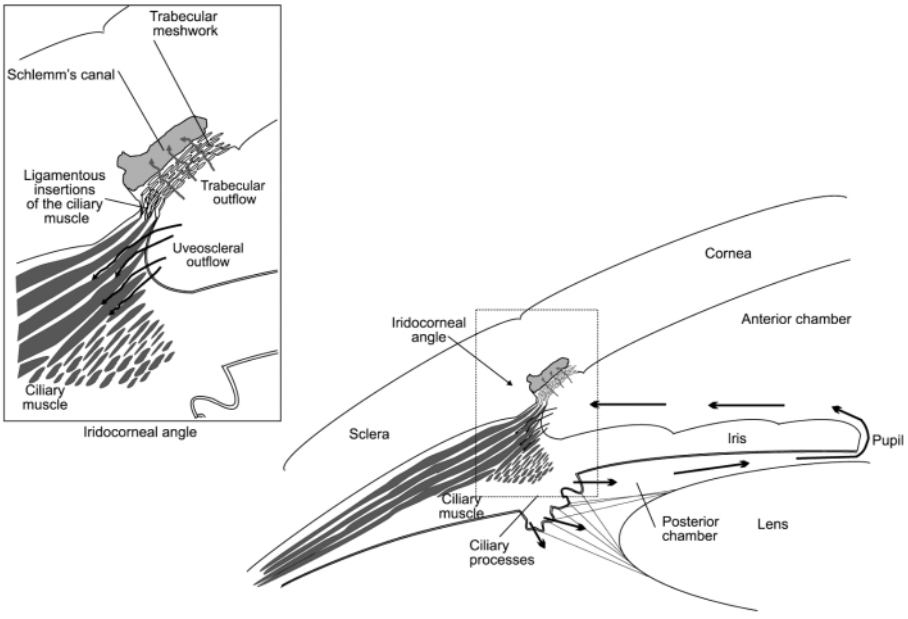

FIGURE 1. Schematic diagram of the aqueous humor cycle. The aqueous humor is formed in the ciliary processes from arterial blood. It is secreted to the posterior chamber and reaches the anterior chamber by crossing the pupil. The *inset* shows the outflow pathways. Aqueous humor exits the anterior chamber via two routes: the uveoscleral or nonconventional outflow pathway and the trabecular or conventional pathway, which comprises the trabecular meshwork (TM) and Schlemm's canal. The balance between the two routes is established by the ciliary muscle tone. More fluid leaves the eye via the uveoscleral pathway when the ciliary muscle is relaxed than when it is contracted.

Schlemm's canal is the last barrier that aqueous humor has to cross before exiting the eye. Because of the high density of pores across its surface, theory estimates that this layer provides of 10% of the total resistance in the conventional outflow pathway (2).

On the basis of electron microscopy studies, it has been proposed that aqueous humor mainly crosses the inner wall endothelium of Schlemm's canal by two different mechanisms: a paracellular route through the junctions formed between the endothelial cells (5) and a transcellular pathway through intracellular pores of the same cells (10). However, the functional importance of each of these two pathways is still unclear.

Although the anatomic organization of the TM already suggests unique regulatory properties of this structure in relation to the outflow of aqueous humor, we should also consider the significance of the ligamentous insertions of ciliary muscle into the cribriform region of the TM (15). Finally, we should also be aware that the TM has autonomic and sensory innervation (16), which may release different neurotransmitters to modulate TM permeability.

The description provided above is valid for primates and humans, but the existence of differences with other mammals commonly used as experimental models, such as rabbits or cows, must be taken into account. For example, in these two species the TM shows a lower degree of organization and a venous plexus replaces Schlemm's canal (10).

Functional properties of TM cells

The introduction of TM primary cell cultures constituted an important tool to study the physiology and pharmacology of TM cells. Despite all of the possible drawbacks of these preparations (i.e., receptor up- or downregulation), they have provided an invaluable tool to characterize pharmacological properties of TM cells. Specific receptors for neurotransmitters and neuropeptides, including epinephrine, acetylcholine, and neuropeptide Y, have been identified in TM cells, indicating that they can detect the activity of sensory and autonomic fibers innervating the tissue. In addition, a long list of vasoactive peptides and growth factors (e.g., endothelin-1, bradykinin, etc.) trigger intracellular signaling mechanisms in TM cells. All of these factors are active at very low concentrations (i.e., in the nanomolar range), and it is likely that tissues surrounding anterior and posterior chambers secrete these substances, which might control TM function in a paracrine manner. For example, it has been documented that the nonpigmented cells of the ciliary body secrete substances to the aqueous humor such as atrial natriuretic peptide, endothelin-1, or galanin that could activate their specific membrane receptors in TM cells [reviewed by Coca-Prados et al. (3)].

However, due to the heterogeneity of the TM, the techniques used in tissue culture sometimes produce conflicting results. A way to overcome the problem of TM tissue heterogeneity is to establish primary cultures limited only to the juxtacanalicular and corneoscleral regions of the TM (18). These authors showed that the expression of αB-crystallin (a major protein component of the mammalian eye lens and a member of the small heat shock protein family with chaperone-like function) is enhanced in juxtacanalicular vs. corneoscleral cells. The use of gene arrays showed that in response to an increased IOP, TM cells upregulated the αB-crystallin gene, among other genes such as α-tubulin, collagenase, etc. probably involved in vascular permeability, secretion, extracellular matrix remodeling, cytoskeleton reorganization, and scavenging of reactive oxygen species (8). However, it is not known how TM cells detect IOP changes or how this detection is coupled to the changes in gene transcription mentioned above.

In this regard, a group of proteins of particular interest are the ion channels of TM cells, because mechanical stretch can modify the activity of some them, such as the high-conductance Ca^{2+}-activated K^+ channel (BK_{Ca}) (7, 19). This channel increases its open probability when pressure is increased (7).

Therefore, the stretch of cell membrane by a pressure rise leads to a K^+ efflux from cell cytosol and a decrease in cell resting membrane potential. It remains to be elucidated whether this activity might affect gene expression or other cell activities. However, if TM cells are regarded as contractile, this would mean that cells relax when pressure rises. Similarly, volume-regulatory properties would be affected as well. However, because of the cellular heterogeneity of the tissue, it is difficult to translate a single-cell observation to tissue function.

Other ion channels characterized in TM cells have been the L-type Ca^{2+} channel (19), the inwardly rectifying K^+ (K_{ir}) 2.1 channel (11), and swelling-activated Cl^- channels (13). These channels can be involved in several functions ranging from volume-regulatory responses to cell contraction. All of these channels are potential targets of many neurotransmitters and hormones. They are also susceptible to being modulated by intracellular mediators (i.e., cAMP, cGMP, nitric oxide). TM cells show a variable expression of these channels. For example, only a fraction of cultured cells express the K_{ir} 2.1 channel, whereas the BK_{Ca} channel is widely expressed.

Mechanisms governing TM permeability

The ligamentous insertions of the ciliary muscle in the TM (15) modulate the permeability of this tissue to aqueous humor. When the ciliary muscle contracts, its insertions widen the intercellular spaces in the TM and the permeability of the tissue increases. Simultaneously, uveoscleral outflow decreases. In the opposite scenario, when the muscle relaxes, the intercellular spaces of the TM become narrower and the trabecular outflow is subsequently reduced. Correspondingly, the uveoscleral outflow is increased (Fig. 1). Therefore, the aqueous humor outflow is distributed between the trabecular and uveoscleral pathways depending on the tone of the ciliary muscle [reviewed by Wiederholt et al. (2)]. Drugs mimicking parasympathetic nerve stimulation, which contracts the ciliary muscle, increase the amount of aqueous humor drained through the conventional outflow pathway. This effect was characterized by the response to pilocarpine (a muscarinic agonist) in in vivo experiments performed in monkeys. Only when the ciliary muscle was inserted in the TM was pilocarpine able to increase outflow facility. The drug lost its effect when the muscle was surgically excised.

However, other results obtained in the experimental model just described showed that there are additional factors influencing the permeability of the TM. For example, drugs like epinephrine relax the muscle through activation of β_2-adrenoceptors but, contrary to expectations, they increased trabecular outflow (10) and thereby are used in antiglaucoma therapy. Along the same line, it has been shown that when the ciliary muscle is excised from the TM, bradykinin and serotonin decrease outflow facility. Bradykinin also shows the same effect in bovine and human anterior eye segments perfused in vitro at a constant pressure (12).

So, in addition to the ciliary muscle, what are the other modulators of TM permeability? Experiments performed with bovine eyes have shown that the TM is a contractile tissue itself, with properties similar to smooth muscle. It contracts when exposed to muscarinic agonists, α_1-adrenergic agonists, and endothelin-1 and it relaxes when β_2-adrenergic agonists, L-type Ca^{2+} channel blockers, or nitric oxide donors are applied. For example, TM contractility is linked to Rho kinase A, which can be regulated by PKC isoforms and does not require Ca^{2+} for its activation (Rho kinase A inhibits myosin phosphatase, resulting in an accumulation of phosphorylated myosin light chain, which is then capable of interacting with actin to produce contraction). The contraction of the TM cells decreases the permeability of the TM because the size of the intercellular spaces is reduced. Similarly, when TM cells relax, the opposite effect appears and the permeability of the tissue increases. A model based on a functional antagonism between the tones of the ciliary muscle and the TM has been proposed [reviewed by Wiederholt et al. (19)] because the TM seems to have particular contractile properties. This model would explain how β-

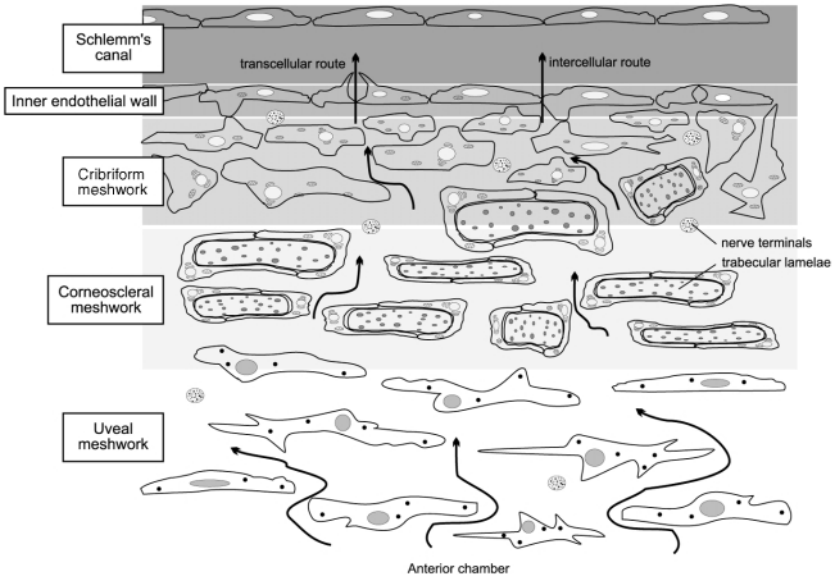

FIGURE 2. Schematic diagram of the TM. The arrows indicate the direction of the aqueous humor, from the anterior chamber toward Schlemm's canal. The different regions of the TM are the uveal meshwork, corneoscleral meshwork, juxtacanalicular or cribriform meshwork, inner wall of Schlemm's canal, and Schlemm's canal. Aqueous humor flows through the intercellular spaces of the TM and crosses the inner wall of Schlemm's canal via two different mechanisms: an intercellular route and a transcellular route. Resistance to aqueous humor flow increases progressively from the anterior chamber to Schlemm's canal as intercellular spaces narrow.

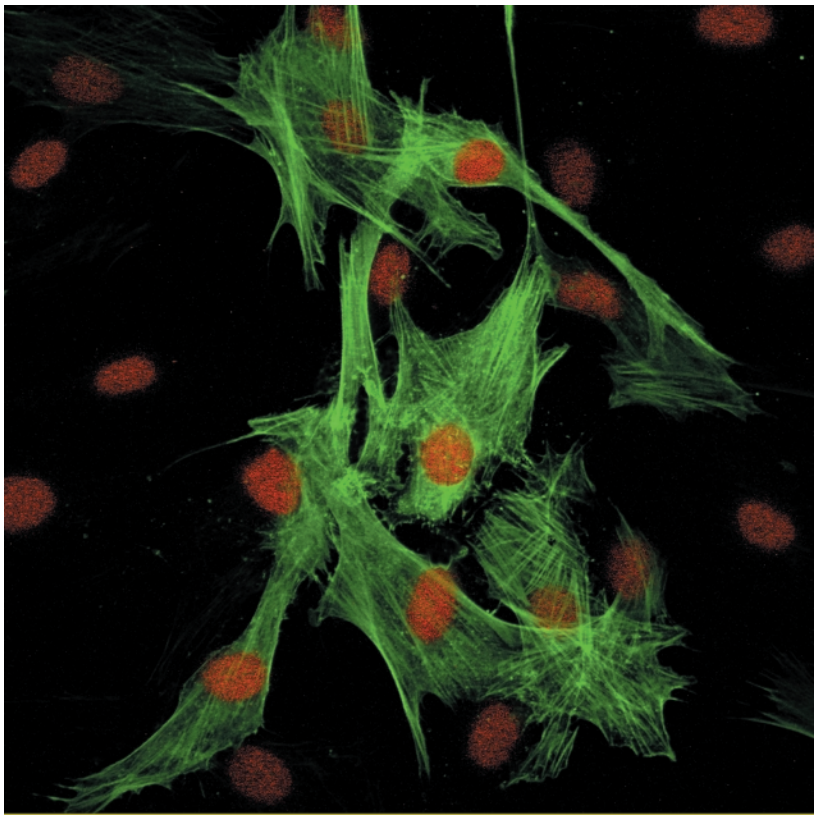

FIGURE 3. Heterogenous expression of α-smooth muscle actin (green) in TM cells in culture. The nuclei of cells were stained with propidium iodide (red).

adrenergic agonists used in glaucoma treatment (i.e., epinephrine), by activating β_2-adrenoceptors, relax the TM and, as a result, increase aqueous humor outflow. Nonetheless, the main action of adrenergic agents used in the treatment of glaucoma, such as timolol, a β_2-adrenergic blocker, is to decrease IOP due to a reduction of aqueous humor inflow.

The observations about the contractility of the TM made in intact and in vitro preparations have been confirmed and extended in tissue culture. Actually, only a small proportion of TM cells is stained for contractile markers such as α-smooth muscle actin or myosin (4) (Fig. 3). It is possible that, due to the heterogeneity of the tissue, not all of the cells are capable of contraction.

Regulatory volume responses of TM cells influence the tissue permeability. By using an in vitro system of anterior chamber perfusion in which the ciliary body had been removed, it was demonstrated that changes in the osmolality of the perfusate modified the permeability of the TM. For both calf and human species, hyperosmotic solutions increased and hyposmotic solutions decreased outflow facility, respectively (1, 9). In this regard, it should be noted that: *1*) TM cells present a robust Na^+-K^+-$2Cl^-$ cotransporter whose activity is modulated by several neurotransmitters and hormones (14), such as norepinephrine, which reduces cotransport activity, or vasopressin, which increases it; *2*) TM cells show a regulatory volume decrease mechanism in response to hyposmotic challenge mediated by swelling-activated Cl^- channels and a K^+-Cl^- symport (13); and *3*) TM cells express aquaporin-1 water channels that can modulate resting cell volume (17). Are all of these volume-regulatory mechanisms related to outflow facility? Aquaporin-1 knockout mice do not appear to show any alteration in the outflow facility rate (20). Also, block of the Na^+-K^+-$2Cl^-$ cotransporter by bumetanide does not change outflow facility in human or primate eyes (6). However, the role of other mediators of volume-regulatory responses, such as the Na^+-H^+ antiport (13), needs to be elucidated.

Besides the effect of ciliary muscle contraction, the contractility of the TM per se, and the volume-regulatory responses of TM cells, other properties of TM cells can also modulate outflow facility. One example is the balance between synthesis and degradation of extracellular matrix. Because this process involves mechanisms of protein expression, it is unlikely to be involved in a short-term effect (as would be the case for contraction), but it is probably very important in shaping the structure of the TM throughout life. In fact, with age, resistance to aqueous humor outflow increases, TM cell numbers decrease, and alterations in the extracellular matrix in the juxtacanalicular region occur. These changes produced by age resemble the changes found in glaucomatous eyes compared with age-matched normal eyes. As already described, the TM is a structure with a high content of extracellular matrix, and TM cells can synthesize a great variety of extracellular matrix proteins as well as matrix metalloproteinases (MMPs). The sustained expression of MMPs by TM cells might contribute to the maintenance of an adequate extracellular matrix composition and therefore a correct resistance to outflow. Finally, some aspects of TM physiology and their significance to tissue permeability are still unknown,

such as the strong phagocytic activity of TM cells, a γ-interferon-modulated process.

Toward a new generation of antiglaucoma drugs?

The TM can be considered a filter of the aqueous humor whose pore diameter changes due to the combined action of an extrinsic factor, the tone of the ciliary muscle, and several intrinsic factors, such as contractility, cellular volume, and extracellular matrix status. One of the main problems in current glaucoma therapy is the lack of specificity of antiglaucoma agents. After topical application (eye drops), drugs reach almost all ocular tissues. Moreover, in the particular case of aqueous humor dynamics, pharmacological receptors involved in aqueous humor formation are also related to aqueous humor drainage (i.e., α-adrenergic receptors). This fact, together with the heterogeneity of the tissue, creates a challenging scenario for the development of new drugs that specifically target the function of the TM. Also, it cannot be forgotten that many of the preliminary experiments were carried out in nonprimate species, and because of the anatomic differences between animal and primate/human eyes, many of the findings must be taken cautiously.

At this point, it is necessary to define which mediators of the already-described intrinsic activities of the TM have suitable pharmacological profiles. Although we are far from such a definition, recent findings are promising. An example is the use of the inhibitor of the Rho-associated protein kinase Y-27632, which increased outflow facility in bovine eyes. Although the aquaporin-1 knockout did not show alterations on outflow, the design of drugs able to reduce the resting cell volume of TM cells by targeting ion channels or transporters specifically expressed in this tissue is still a promising new approach. In this direction, it would be very useful to design future experiments to discover ion channels or transporters uniquely expressed in the TM.

In conclusion, the TM is a tissue whose permeability is acutely modulated by the combined action of the ciliary muscle tone together with its own contractile and volume-regulatory properties. A better understanding of how endogenous hormones and neurotransmitters balance TM activity would permit the development of more efficient strategies to tackle glaucoma.

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